

Potassium Ion Chemical Ionization and Other Uses of an Alkali Thermionic Emitter in Mass Spectrometry

Daniel Bombick, J. David Pinkston, and John Allison*

Chemistry Department, Michigan State University, East Lansing, Michigan 48824

Certain solids when heated and positively biased emit ions. Here, an aluminosilicate matrix containing K_2O is used as a source of gaseous potassium ions. A simple method is presented for introducing these emitters into the CI source of a mass spectrometer. K^+ adds to most compounds containing π - or n -donor sites to produce an $(M + K)^+$ ion. Some compounds, notably amines, react on the hot surface to produce ions. The behavior parallels the response of thermionic ionization GC detectors. When nonvolatile compounds are deposited on the emitter, ions representative of the compound are formed. This is demonstrated for several di- and tri-peptides.

When mass spectrometry (MS) is used for chemical analysis, molecular weight, functional group, and structural information can frequently be obtained. Of these, molecular weight information (i.e., the detection of a molecular ion, M^+) is usually the most valuable, because it determines both the elemental composition and the "molecular boundaries into which the structural fragments indicated in the mass spectrum must be fitted" (1). Unfortunately, for a number of reasons, many compounds do not form detectable amounts of the molecular ion following electron impact (EI) ionization (1).

Chemical ionization (CI) is another common technique available to mass spectrometrists (2). Chemical ionization is considered one of the "soft" ionization methods, since it usually induces less fragmentation than does EI, simplifying the mass spectrum. Also, the protonated parent, MH^+ , is frequently formed—i.e., molecular weight information is produced. Munson and Field (3) were the first to demonstrate the analytical applications of CI by using CH_4 as a reagent gas and generated spectra in which the analyte molecules reacted with CH_5^+ and $C_2H_5^+$ (the "reagent ions") in the gas phase. Since this work, many other species have been used as CI reagents. Proton transfer reagents such as H_3^+ will react with most compounds since the proton affinity (PA) of H_2 is low (~ 101 kcal/mol). Isobutene has a much higher PA (195 kcal/mol); therefore $i-C_4H_{10}$ CI only protonates molecules with PA's greater than approximately 195 kcal/mol. Thus, the proton affinity of the species used in CI is the "working variable" which can be adjusted to provide a "universal" or selective ionization method.

Charge exchange (CE) chemical ionization is another CI alternative. In CE, ions such as Ar^+ , N_2^+ , or CO^+ react with analyte molecules by electron transfer. As in proton transfer CI, varying the reagent varies the response—in CE, the variable is not proton affinity, but instead ionization potential.

The popularity of CI is due in part to its compatibility with most MS techniques. Unlike other ionization methods such as field desorption (which usually requires a double focusing magnetic mass spectrometer) CI can be performed with most types of mass spectrometers. Also, CI is compatible with chromatographic inlet systems (i.e., gas chromatography (GC)/MS, liquid chromatography (LC)/MS).

In spite of the many advantages of CI, there are a number of disadvantages. An $(M + 1)^+$ ion is not always observed in CI (as is often the case with alcohols) and a parent ion is not

always observed in CE. Also, it is difficult to catalog CI spectra because they can be strongly pressure dependent. Pressure effects arise in many ways. Frequently, more than one reactive species is generated in CI sources (e.g., CH_5^+ and $C_2H_5^+$ in methane CI, CO^+ and $(CO)_2^+$ in carbon monoxide CE). The relative amounts of these reagent ions vary with pressure. Also, product ions can be collisionally stabilized at high pressures. Thus, small pressure changes can dramatically affect the number and relative intensities of ions in a CI mass spectrum.

In part, this paper discusses the use of metal ions, here K^+ , as CI reagent ions. Studies presented here show that K^+ reacts with most types of molecules (M) to form the "pseudomolecular ion" $(M + K)^+$; i.e., molecular weight information is easily obtained. By the technique described, metal ions can be generated (with a selected kinetic energy) in the absence of corresponding neutrals—providing the basis for a relatively low pressure CI method.

Recently, a large body of literature has appeared concerning the gas phase chemistry of metal ions with organic molecules. Both transition-metal and alkali ions have been studied (4-6).

Transition-metal ions can be generated in the gas phase by EI on organometallic compounds (7) or by laser desorption/ionization from solid surfaces (8). The chemistry of gaseous transition-metal ions with organic molecules is rich, often producing products which provide both molecular weight and structural information.

In contrast, alkali ions generally react by simpler mechanisms (4-6). Ions such as Li^+ form electrostatic complexes with many analytes such as π donor bases (e.g., benzene) and n -donor bases (e.g., ethers). Adduct ions (also referred to as "cationized molecular ions") of the type $(M + Na)^+$ have also been reported (4-6). Alkali metal ions appear to be useful CI reagents for obtaining molecular weight information due to the low IP's of these metals. In the case of the $(M + K)^+$ adduct, for example, the charge presumably remains localized on the metal. Thus, the decomposition pathway with the lowest activation energy is simply the loss of K^+ , instead of the fragmentation of the attached molecule. The probability of a metal induced reaction relative to attachment appears to decrease as the metal is varied from Li^+ to K^+ (4-6).

In 1936, Blewett and Jones (9) described a convenient method for producing metal ions in the gas phase. Metal ions are produced by heating and biasing a glass or metal oxide. The glasses are usually metal oxides in an aluminosilicate matrix. Oxides such as In_2O_3 behave similarly. (Such solids which produce ions on heating are referred to as thermionic emitters.) Such sources were reported for 17 metal ions. Those for alkali ions are particularly copious emitters.

This paper will focus on uses of thermionic sources of K^+ ions. We present a simple, low cost method for producing variable energy metal ions for use as CI reagents. The method is adaptable to any mass spectrometer with a CI source and direct inlet probe. In addition, alternate modes of operation are presented which will provide additional dimensions of mass spectral information.

EXPERIMENTAL SECTION

All experiments were performed on an unmodified Hewlett-Packard 5985 GC/MS/DS, equipped for manual tuning as de-

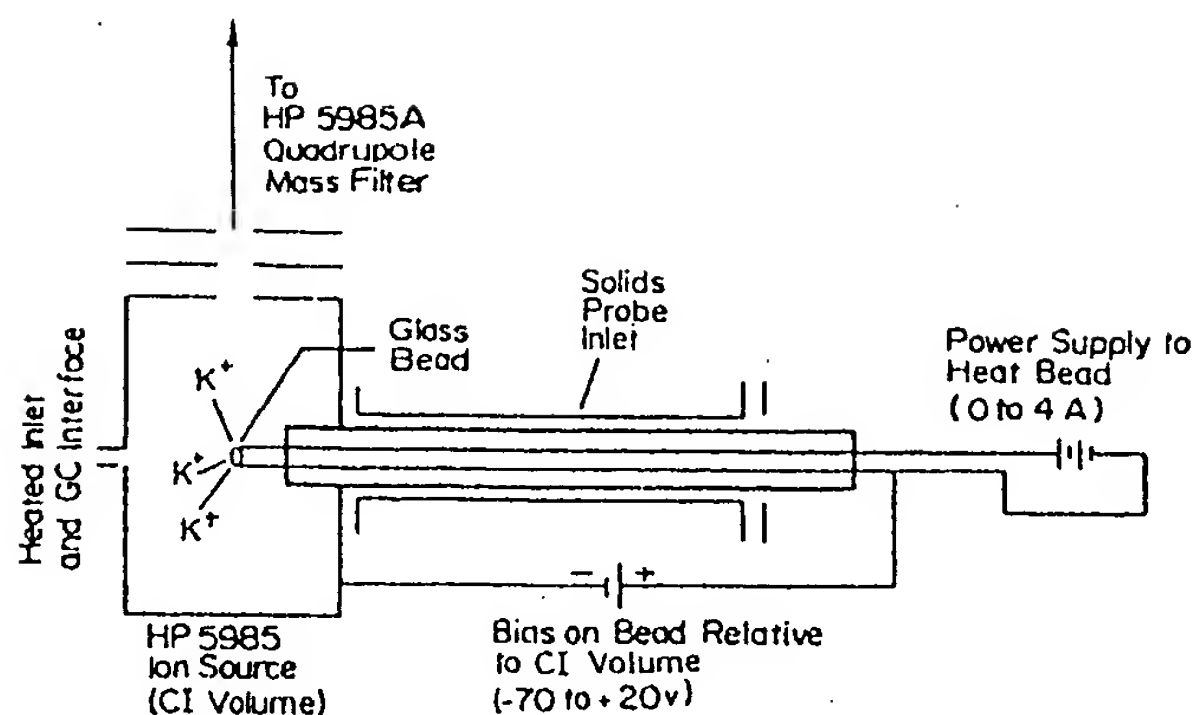


Figure 1. Schematic diagram of thermionic emission probe in the mass spectrometer's ion source.

scribed elsewhere (10). Thermionic emitters were mounted on a fabricated probe assembly and inserted into the CI volume of the ion source through the direct insertion probe inlet (see Figure 1). The tip of the thermionic emitter was made by inserting 0.007 in. (0.178 mm) rhenium wire through a 0.5 in. (1.30 cm) length of two-holed ceramic rod of 0.047 in. (1.19 mm) diameter. The ceramic rod gives mechanical stability to the probe tip and insulates the rhenium wire from the ion source. A small loop of the rhenium wire extends from one end of the ceramic rod. The two free ends of this wire are spot-welded onto insulated leads housed within the probe assembly.

"Thermionic K^+ glass" has the composition $1 K_2O:1 Al_2O_3:2 SiO_2$. Its preparation has been described elsewhere (9). The wire loop on the probe tip is dipped into a molten mixture of the thermionic glass, withdrawn, and allowed to cool. This procedure produces a bead of glass (approximately 0.030 in. (0.76 mm) in diameter) on the wire loop. This bead is small enough to fit through an existing hole in the CI source volume and was sufficient for at least 40 h of continuous operation.

A 0–4 A, low-voltage power supply is used to resistively heat the thermionic glass bead on the wire loop. A 0–20 V, low-current power supply biases the bead positively with respect to the CI volume. Biases from 0 to +20 V were used in these studies. For EI studies using the K^+ CI probe, the emitter was biased from –20 to –70 V with respect to the CI volume. A current of 1.5–2.0 A through the wire is sufficient to induce thermionic emission from the glass.

The GC oven was used as a heated gaseous batch inlet. Most samples were introduced through the "capillary direct" port of the GC/MS interface. Typical pressures in the CI volume were between 20 and 700 mtorr as measured by a thermocouple gauge mounted on a stainless steel tube which was brought into contact with the CI volume through the direct insertion probe inlet. The GC studies were performed with a 6 ft \times $1/16$ in. (1.8 m \times 1.6 mm) glass column packed with 3% OV-101, interfaced to the ion source through the jet separator. Helium was used as a carrier gas at a flow rate of 30 mL/min. Temperature programmed runs (50–100 °C, 5 °C/min) were used.

Experiments involving direct introduction of nonvolatile samples on the probe were performed by using a saturated solution in methanol, depositing a drop on the glass bead, and drying. In these experiments, it was found that a thin coating of glass on the rhenium wire worked better than a larger glass bead.

All (commonly available) chemicals were ACS reagent grade and were used without further purification. All peptides were obtained from Sigma Chemical Co. The crown ethers were obtained from Aldrich Chemical Co. All were used without additional purification except for outgassing liquid samples with several freeze-pump-thaw cycles.

RESULTS AND DISCUSSION

Results of K^+ CI experiments are presented here. Also, a number of alternate uses of the K^+ emitter probe will be suggested.

When the probe as described is inserted into the CI volume of the mass spectrometer, heated, and biased, K^+ ions are

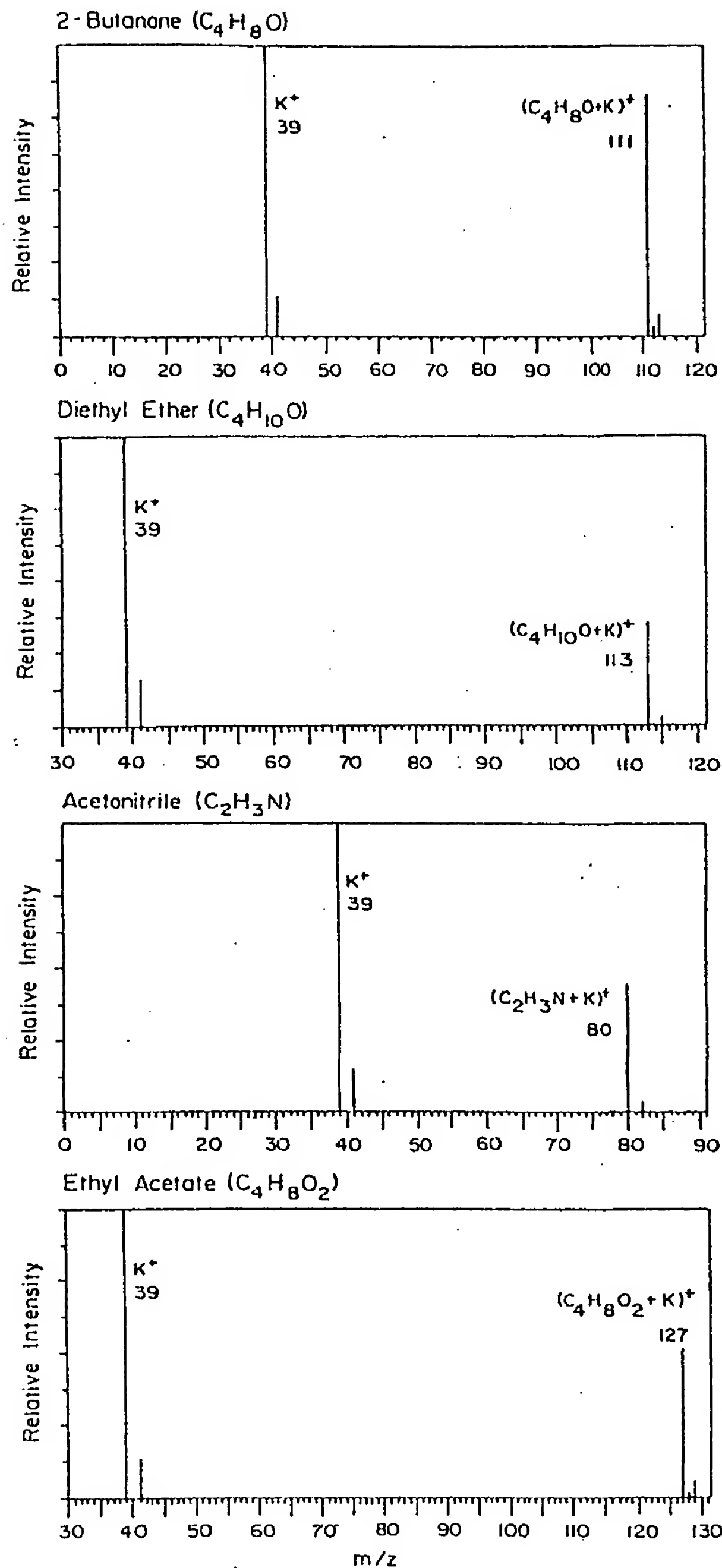


Figure 2. K^+ CI spectra of compounds representative of four organic functional groups.

formed with no apparent rise in pressure. In addition to the dominant K^+ peak in the mass spectrum, small amounts of Na^+ , Cs^+ , and Rb^+ are formed.

In most cases, when organic vapors are admitted into the CI volume, only $(M + K)^+$ ions are formed. Most n- or π -donor bases produce especially intense cationized species. The K^+ addition process is kinetic energy dependent. Addition products are observed when the K^+ emitter bead is biased relative to the CI volume only in a "bias window" ranging from 0.5 V to approximately 5 V. The exact value of the upper voltage cutoff depends on the particular compound. Figure 2 shows K^+ CI spectra for molecules representative of four organic functional groups. Of the more than 30 compounds studied, K^+ CI produced $(M + K)^+$ as the only product, except

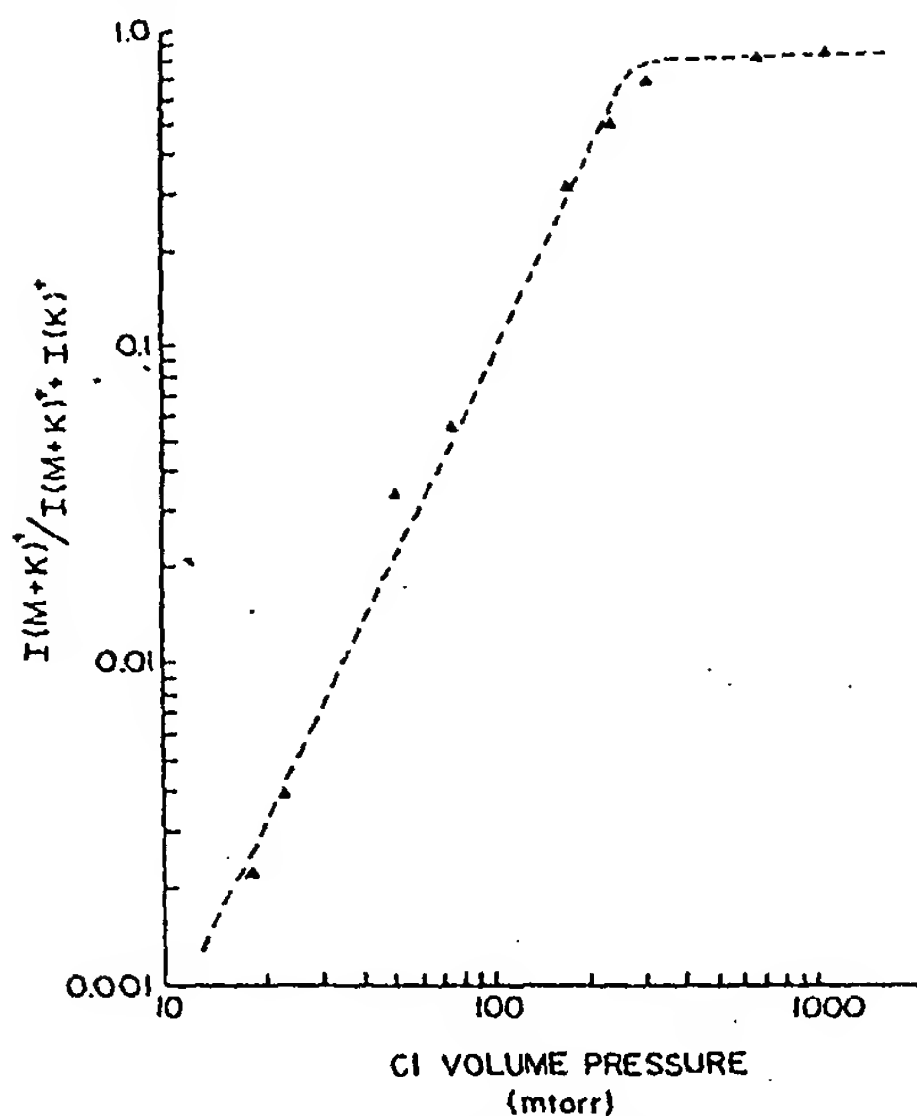


Figure 3. A log-log plot of $I(M+K)^+ / [I(M+K)^+ + I(K)^+]$ vs. pressure of acetone (I refers to intensity).

in a few cases. No products were formed with cyclohexane. Alkylamines and 2-propanol give more complex spectra. On the basis of these observations, K^+ CI may be superior to other CI techniques for obtaining molecular weight information.

The formation of an addition product in the gas phase has been reported to occur in a bimolecular process, if the complex can in some way lose its excess energy (e.g., emit a photon) (11). Addition is more commonly assumed to be a three-body process, in which a neutral molecule collides with an ion-molecule complex and removes an amount of energy, stabilizing the $(M+K)^+$ complex. Ketones gives abundant $(M+K)^+$ ions in their K^+ CI spectra. Figure 3 shows a log-log plot of $I(M+K)^+ / [I(M+K)^+ + I(K)^+]$ vs. pressure of acetone (in the CI volume). Apparently, the process leading to the formation of $(\text{acetone} + K)^+$ is a reaction which is second order in acetone. At the higher pressures, the K^+ concentration is reduced substantially and the apparent order begins to change. Thus, at least for acetone, the reaction for K^+ CI can be written as



The termolecular nature of the adduct formation which was observed is consistent with the previously reported kinetics of alkali ion adduct formation (12). Based on this, K^+ CI would be very suitable for GC/MS without a splitter, since no response for carrier gas molecules will be seen, and the carrier gas can act as the third body in the analyte addition reactions with K^+ .

Amines gave richer K^+ CI spectra than the other compounds studied. Figure 4 is an example of a typical K^+ CI spectrum of triethylamine. In this particular spectrum triethylamine exhibited no $(M+K)^+$ ion. The ions at m/z of 102, 100, 86, and 72 can be identified as $(M+H)^+$, $(M-H)^+$, $(M-CH_3)^+$, and $(M-C_2H_5)^+$, respectively. The product ions in the K^+ CI spectrum are also the major ions in the EI spectrum of this compound. This fact, along with the fact that, of the compounds studied, amines had the lowest ionization potential, led us to believe that a charge transfer process was occurring (13). That is, the mechanism could be written as follows:

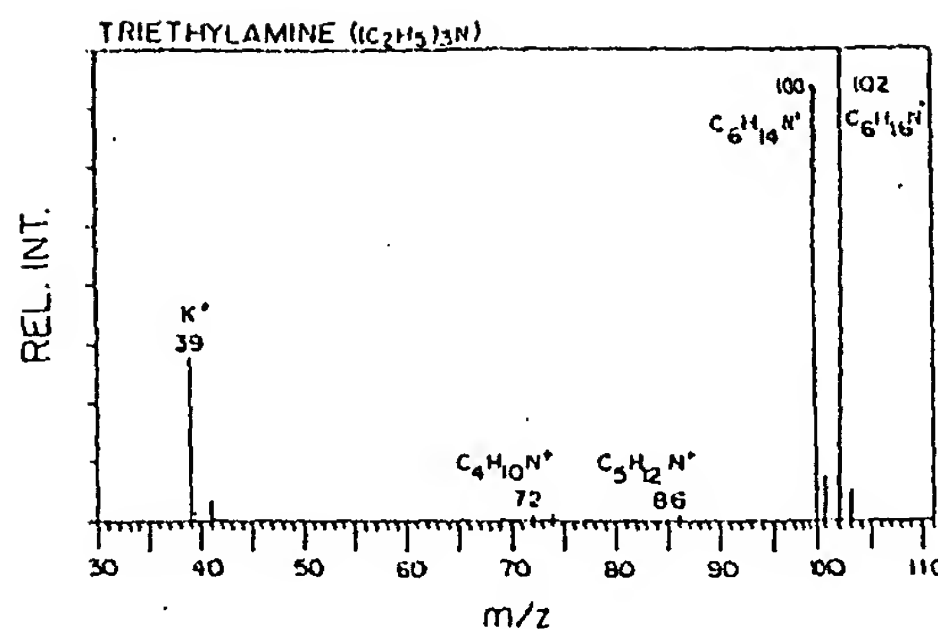
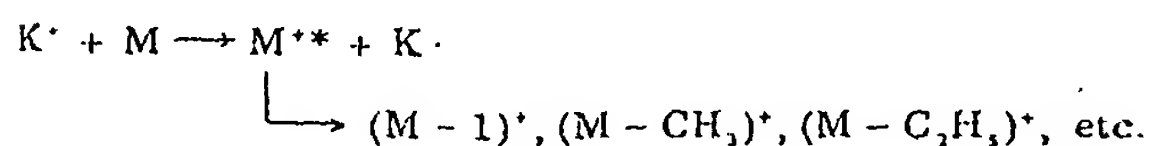


Figure 4. K^+ CI (surface ionization) mass spectrum of triethylamine. Bead bias is above K^+ addition bias window.

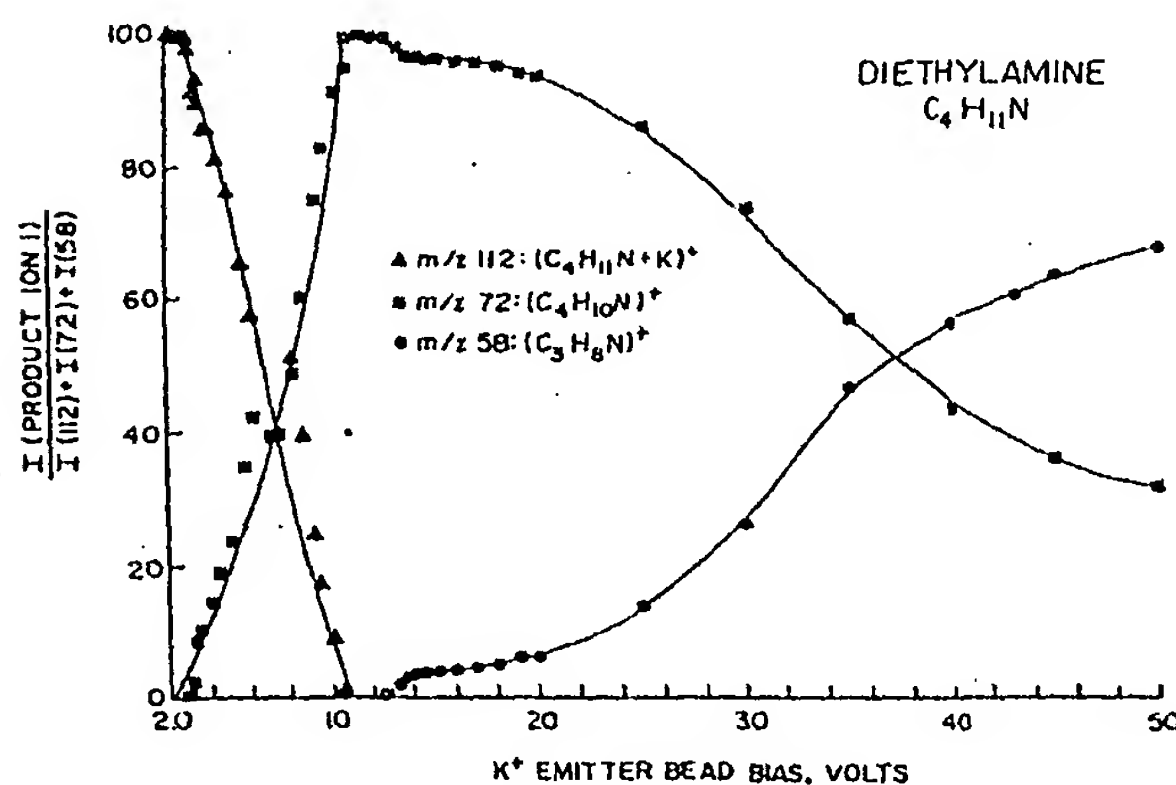


Figure 5. Plot of $I(\text{product ion}) / [I(m/z 112) + I(m/z 72) + I(m/z 58)]$ vs. bead bias for diethylamine. Pressure and heating current were constant at 650 mtorr and 2.0 A, respectively.

Upon further investigation with a bare heated wire probe tip (no glass bead) the same product ions were produced (in contrast to, e.g., acetone, for which no ions are generated from contact with a bare heated wire). In this case, gas-phase potassium ions were absent. Therefore, this suggested that the observed product ions for the amines were a result of surface ionization. Surface ionization of organic compounds on hot surfaces has been reviewed recently (14) and our results parallel those found previously. The formation of the $(M+1)^+$ ion can be attributed at least in part to a gas-phase ion/molecule reaction (presumably of $(M-1)^+$) since the $(M+1)^+ / (M-1)^+$ ratio increases with increasing pressure of the amine.

The products observed for the amines were greatly influenced by the bias voltage applied to the glass bead. The addition product $(M+K)^+$ was seen for all compounds studied at bias voltages between approximately 0.5 and 5 V. However, ions formed due to surface ionization from amines are produced at much higher values of the bead bias. This can be seen graphically in the case of diethylamine in Figure 5. At low voltages the potassium ion adduct of diethylamine is the major product. With increasing bias voltages only the surface ionization products are observed. This bias-dependent behavior can be an advantage as will be shown later.

Surface ionization studies have predominantly been done on pure and oxidized metal surface. In our system, the hot surface consists of the thermionic glass bead (and some exposed rhenium wire). The thermionic glass we use in our probe tip is very similar to the thermionic material contained in thermionic ionization detectors (TID) for gas chromatography. Several surface ionization mechanisms have been proposed for these detectors (15, 16) which account for the observed signal in the presence of nitrogen-containing com-

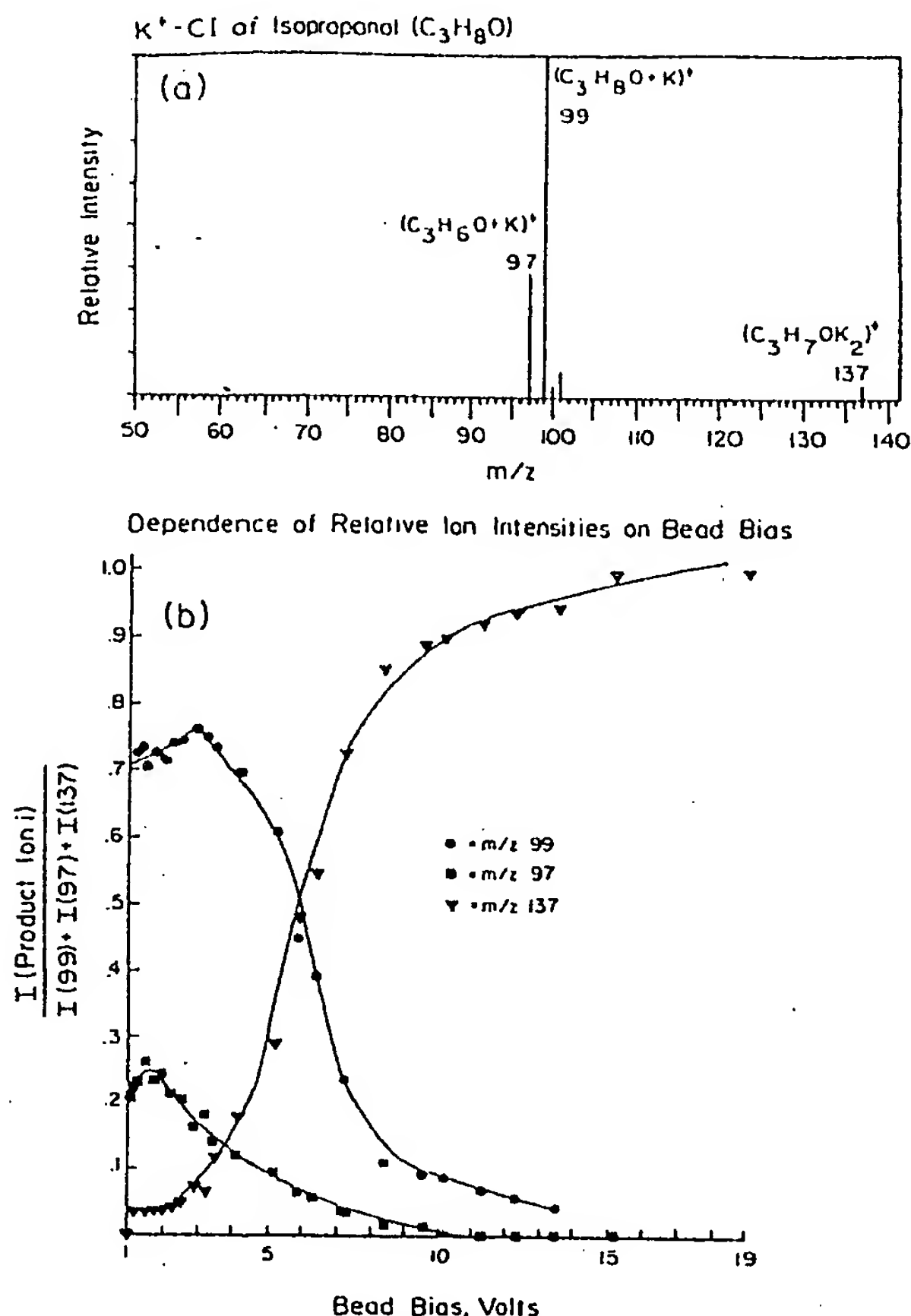


Figure 6. (a) K^+ CI spectrum of 2-propanol with bead biased within K^+ addition bias window. (b) Plot of $I(\text{product ion})/[I(m/z 98) + I(m/z 99) + I(m/z 137)]$ vs. bead bias. Other conditions are the same as those in part a.

pounds. Thus, we are currently studying surface ionization product ions of N- and P-containing molecules with the hot thermionic material in an attempt to postulate a mechanism that explains the behavior of thermionic ionization detectors. (To date, the link between TID response and the large body of literature on surface ionization mass spectrometry has apparently not been made.)

Energy-variable CI reagent ions can be a very useful tool, since it has been demonstrated that ion/molecule reactions can be made to occur (i.e., made exothermic) by the addition of kinetic energy to the reactant ion (17). In the case of K^+ , however, this effect is not observed. We do, however, see dramatic changes in the K^+ CI spectrum for some molecules as the K^+ emitter bead bias varies. Low biases favor adduct formation, $(M + K)^+$. As the bias is raised, the probability for this process dramatically decreases. For many compounds, "fast" K^+ CI gives no products. As was seen for amines, if surface ionization occurs, higher biases are required to remove these ions from the surface. 2-Propanol was found to participate in both gas phase and surface processes. Figure 6a shows a K^+ CI spectrum of 2-propanol (low bias). The predominant ion is the $(M + K)^+$ at m/z 99 and an $(M + K - H_2)^+$ at 97. There is also an ion at higher mass, m/z 137. The m/z 97 and 99 ions decrease as bead bias increases (Figure 6b). The intensity of m/z 137 increases as bead bias increases, typical of a surface process.

An ion of m/z 137 cannot be formed by K^+ and C_3H_7OH in a simple process, since the mass of these two reactants only totals 99 amu. Initially, we identified this ion as $(C_6H_{10}O + K)^+$. That is, by some surface process, a six-carbon species

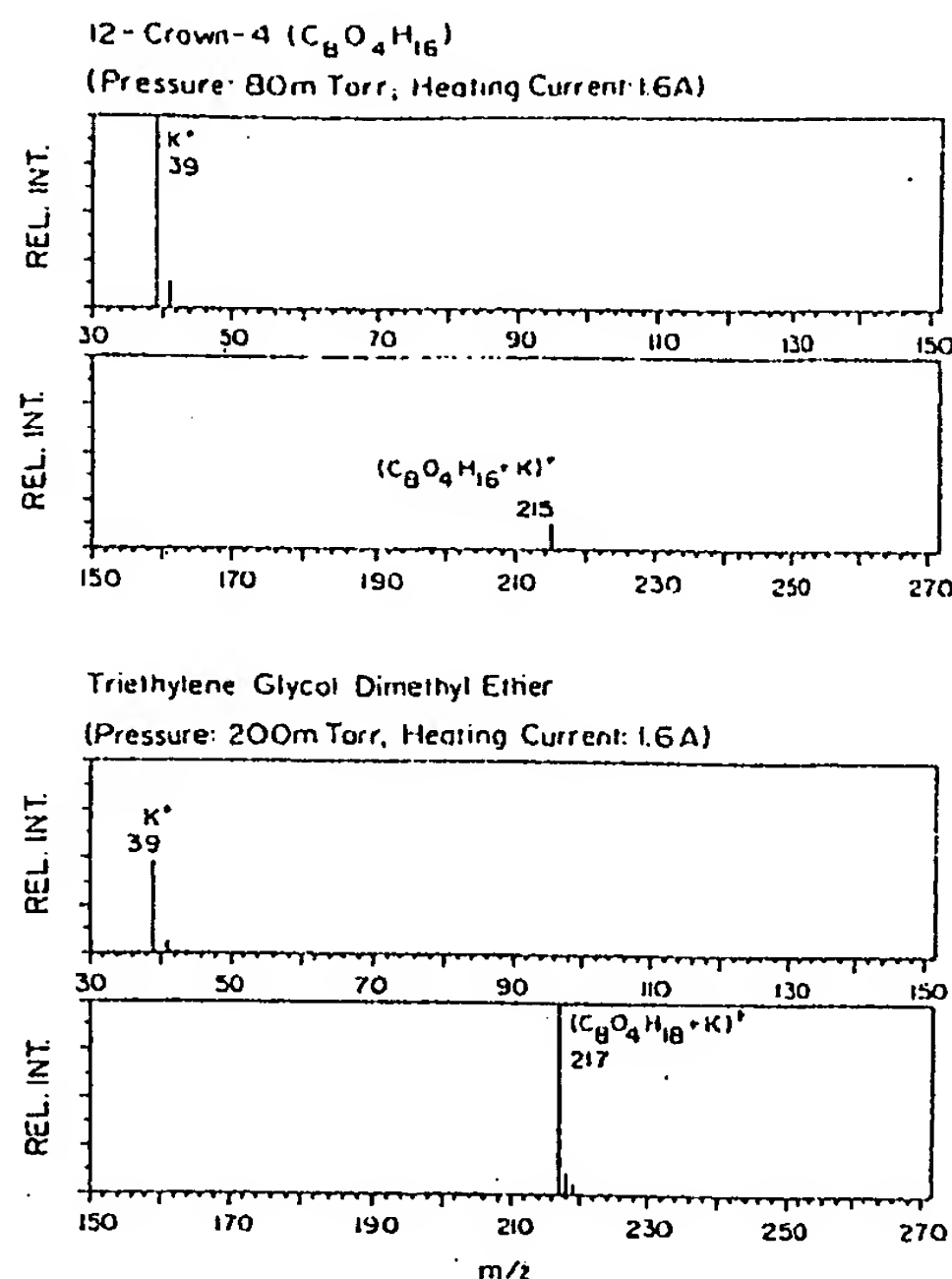
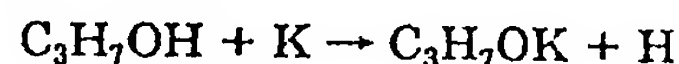


Figure 7. K^+ CI spectra of 12-crown-4 and of triethylene glycol dimethyl ether. Bead bias is within addition bias window in both cases.

was being formed from two propanol molecules. Closer inspection of isotope ratios suggested that m/z 137 contains two potassium atoms, i.e. $(C_3H_7O + 2K)^+$. Presumably some surface process such as



occurs. The C_3H_7OK then complexes with a K^+ ion emitted from the surface. Similar species have been observed in other techniques such as fast atom bombardment (FAB) ionization (18). The "sampling" of species formed on the bead surface by the K^+ ions which are emitted is discussed in more detail in the case of peptide analysis.

In summary, K^+ appears to be useful in providing molecular weight information, except with certain types of compounds, for which ionization on a hot surface is known to be a facile process.

Other Uses of the K^+ Thermionic Emitter Probe in Mass Spectrometry. We are currently developing a number of alternate uses of the probe described in this work. Five alternate uses will be briefly discussed here.

Complexation Studies—Solution Parallels. In solution, crown ethers have proven to be useful species for kinetic and thermodynamic investigations of ion-solvent interactions (19). In the gas phase, such species can be studied in the absence of solvent (20). Crown ethers form strong complexes with alkali ions in solution. With the K^+ emitter, crown- K^+ complexes can also be formed in the gas phase. Figure 7 shows the K^+ CI spectra of 12-crown-4 ($(C_2H_4O)_6$) and its linear analogue, triethylene glycol dimethyl ether $CH_3(OC_2H_4)_3OCH_3$. Studies of competitive ligand exchange of such species may provide thermochemical insights into such metal-ligand complexes.

An Alternating EI/CI Source. In the context of GC/MS, the production of both EI and CI mass spectra from a single sample component is advantageous since both the molecular weight information common in CI and the structural information of EI are available. A number of approaches have been demonstrated. Instruments utilizing dual sources, dual ion beams, and dual detectors have been used. Also, combination

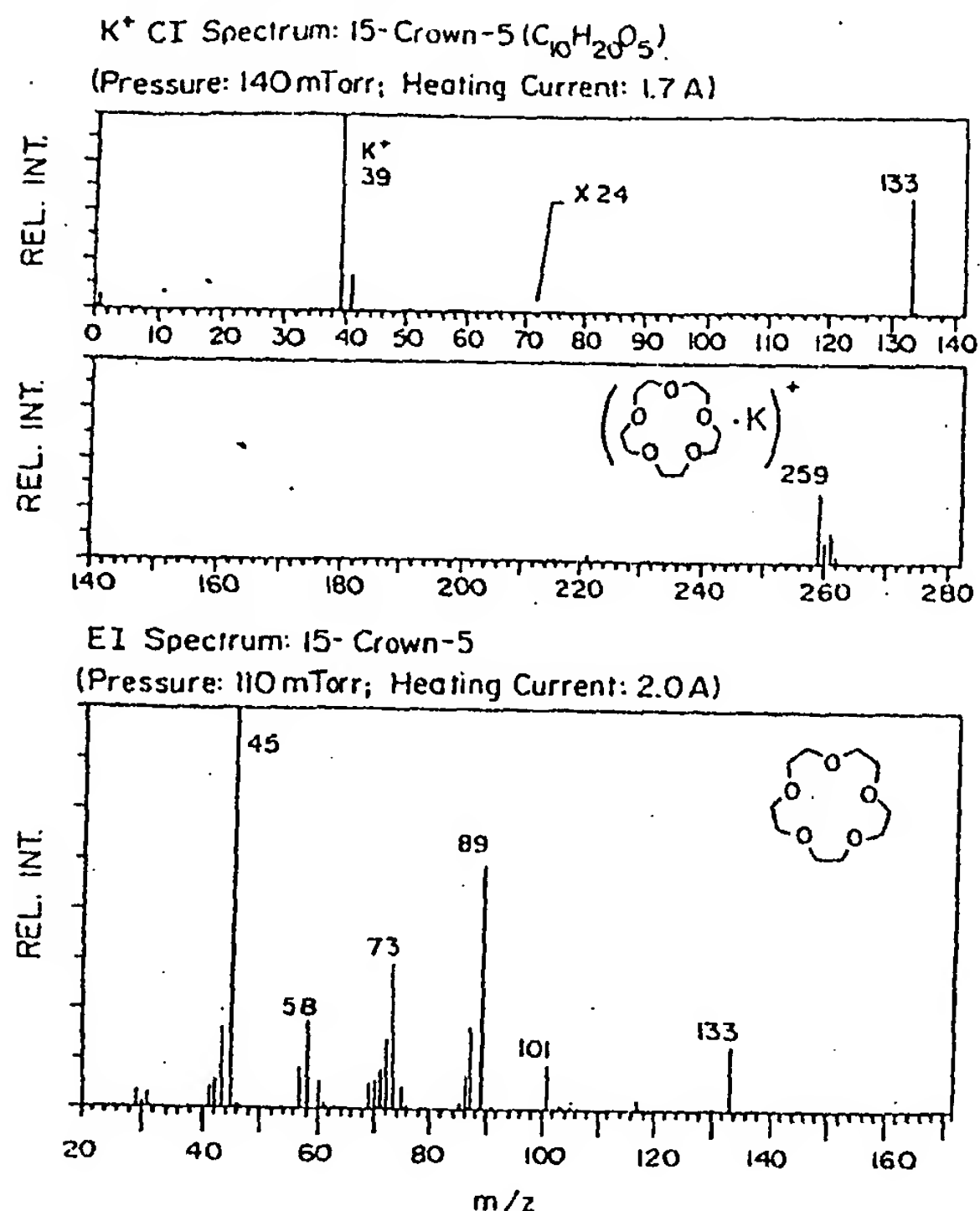


Figure 8. K⁺ CI and EI spectra of 15-crown-5. Bead bias was within the addition window in the first case and was -23 V with respect to the CI volume in the EI spectrum.

EI/CI sources have been used. In the latter, sequential EI and CI spectra can be produced (alternating for each scan).

The K⁺ CI probe, when negatively biased, produces electrons. Apparently, electrons are generated from both the thermionic glass and the rhenium wire when heated. Thus, the bias can be synchronized to the scan flag of the mass spectrometer data system such that, for one mass scan, the bead bias is at, e.g., 2 V (K⁺ CI mode), and the next scan at, e.g., -70 eV (EI mode). To demonstrate this, 15-crown-5 was chosen, since the EI mass spectra of crown ethers are very similar and characteristic of a crown ether, but with essentially no molecular ion to identify the particular crown ether under study. Figure 8 shows EI and CI spectra obtained by using the K⁺ emitter in the CI volume. In an EI mode (here, bias = -23 V) the spectrum agrees well with the expected EI spectrum (21). In the K⁺ CI mode (bias of +2 V), the (15-crown-5 + K)⁺ adduct is prominent, providing the necessary molecular weight information.

This simple technique can extend the information available from a mass spectrometer without costly redesigned sources—in fact, with no instrumental alterations at all.

Operation of an MS in a "TCD" Mode. When a gas chromatograph is coupled to a mass spectrometer, the GC detector is removed in most cases. Many detectors, such as the thermal conductivity detector, are very useful. The thermal conductivity detector (TCD) works on the principle that a hot filament will lose heat according to the composition of its gaseous environment. The concentration of a certain gas in this environment determines the amount of heat loss. The signal produced is proportional to the amount and type of gas surrounding the filament.

The K⁺ emitter can be used to mimic this type of detector response. The intensity of the K⁺ ion signal reflects the temperature of the emitting glass bead. If the total ion intensity (TII) is monitored in a GC/MS experiment, a TCD-like response will be observed. The concentration and thermal

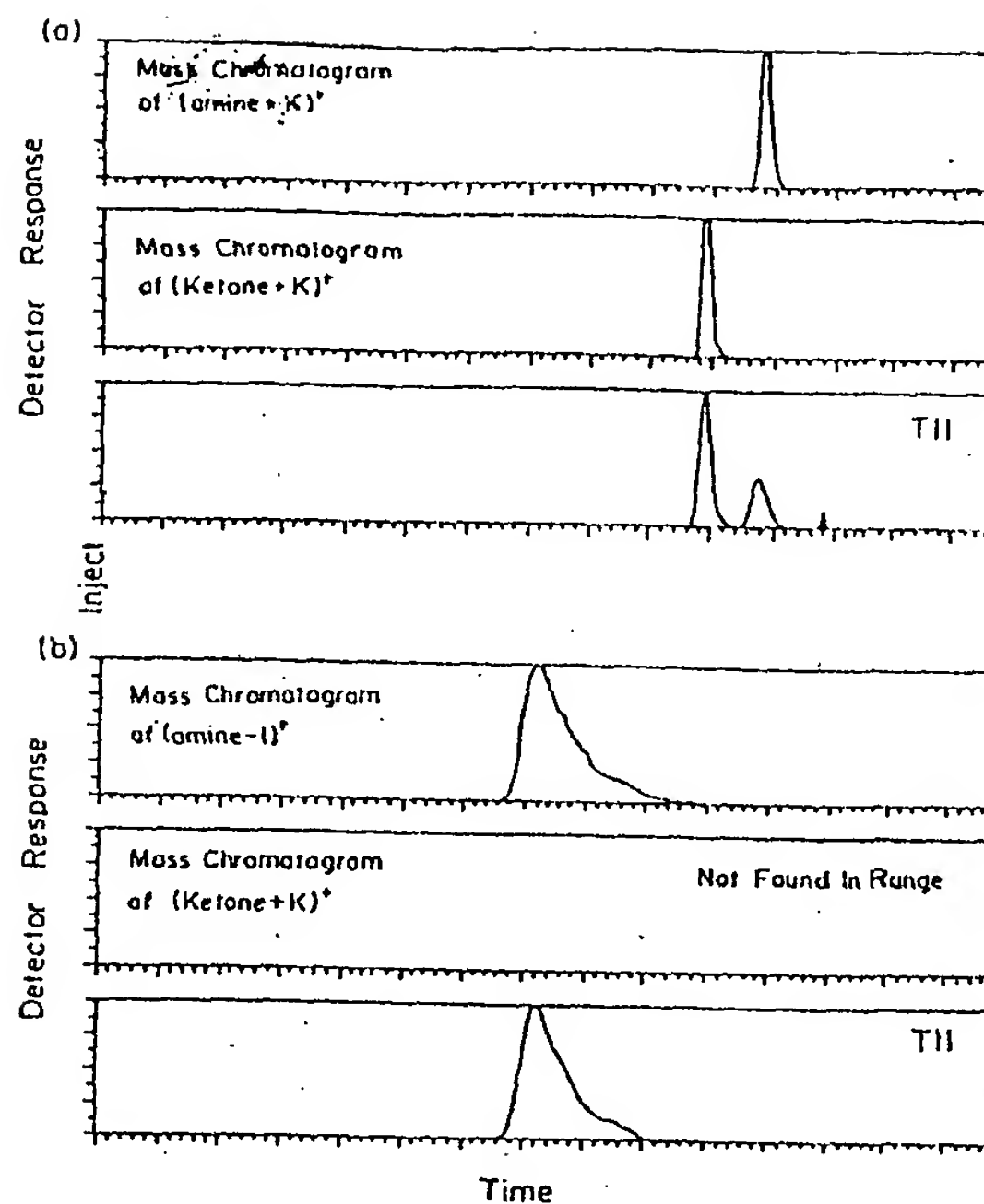


Figure 9. Shown here are mass chromatograms and total ion intensities (TII) obtained from GC/K⁺ CI/MS runs in the analysis of 1 μ L of a 1:1 neat mixture of a ketone and an amine. Mass spectra were collected from m/z 42 to 150. Heating current was 3.0 A. In (a) the bead bias was within the K⁺ addition bias window and addition products of both components are observed. In (b) the bead bias was above the K⁺ addition bias window and the figure shows that only ions from the amine are formed. In (b) the region about the elution times has been expanded. Acetone and di- and triethylamine were used.

conductivities of solute eluting into the CI volume determine to what extent the bead is collisionally cooled. Cooling reduces the K⁺ ion intensity. The response should parallel the conventional TCD. A mass spectrometer is an expensive TCD; however, this option may be preferable to using a second GC which has a TCD in addition to GC/MS for analysis.

Uses of the K⁺ Emitter in GC/MS Experiments. Selective detectors in gas chromatography have proven to be useful in applications such as quantitating compounds which are unresolved by the chromatograph. The bias dependence of the K⁺ CI response can be used as the basis for a "selective" ionization method. In the context of GC/MS, if the K⁺ bead is operating at low bias, (M + K)⁺ ions are observed for most compounds. If the bead is at higher bias, addition will not occur, and only compounds which can react by surface ionization will be detected. This is shown in Figure 9.

Analysis of Nonvolatile Compounds. We have observed that, when a solution containing a nonvolatile compound is evaporated onto the K⁺ glass bead, ions representative of the compound will be generated when the probe is heated. These ions usually last for 10–15 s after the bead is brought up to operating temperature. This technique was investigated by using di- and tripeptides as analytes. We observe that (1) most of the ions formed contain K⁺, (2) (M + K)⁺ is almost always present and is also the highest m/z product formed, and (3) low-energy decompositions of the peptides occur, for example, H₂O and CO₂ loss occurs and an (M + K - CO₂)⁺ or (M + K - H₂O)⁺ is observed.

While a number of possible mechanisms are being considered, a useful working model is as follows: A molecule thermally undergoes a decomposition to two (or more) smaller molecules on the surface. The K⁺ being emitted from the surface beneath these species complexes with these species, "sampling" the molecules present on the hot surface. Figure

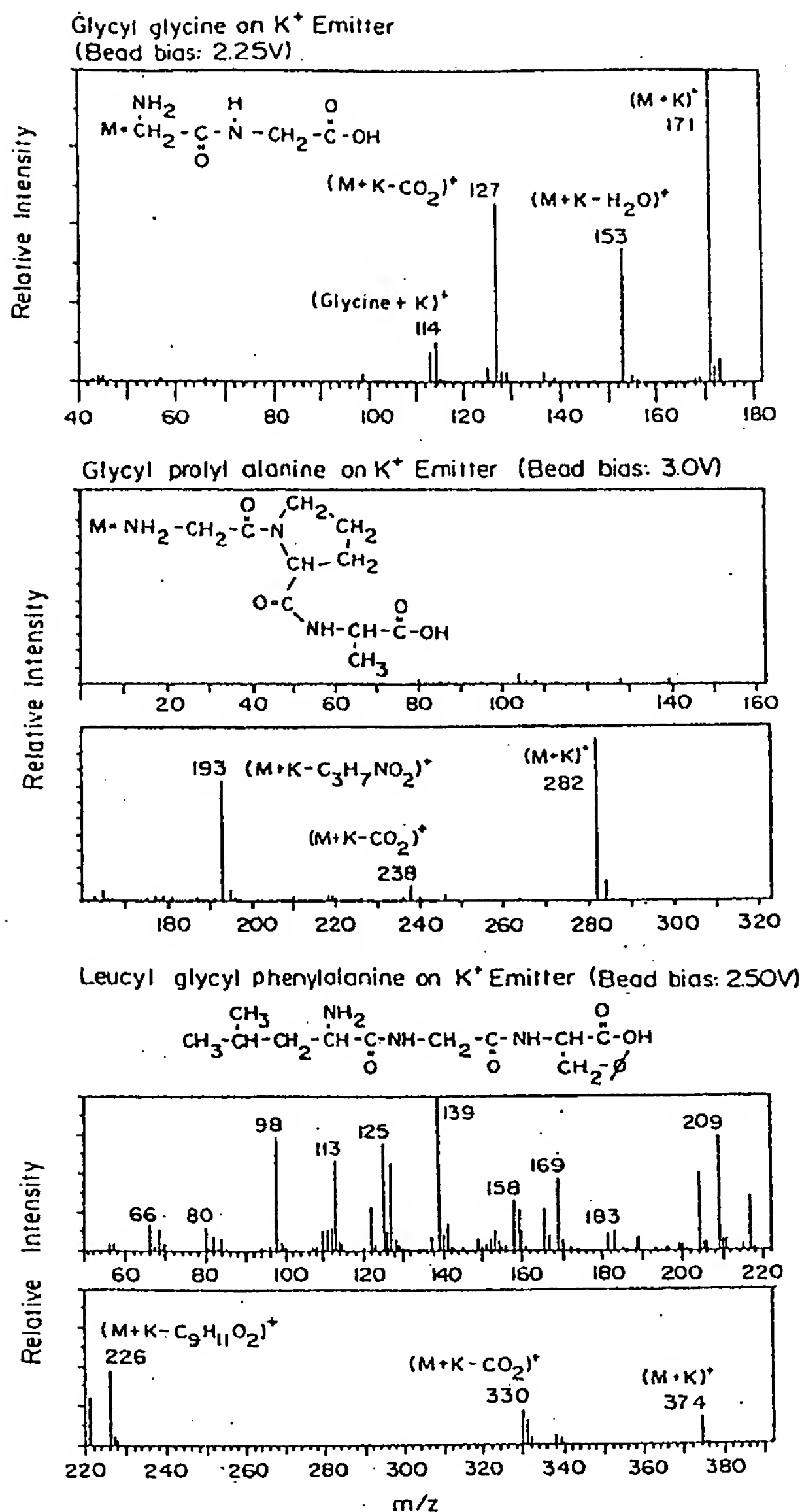
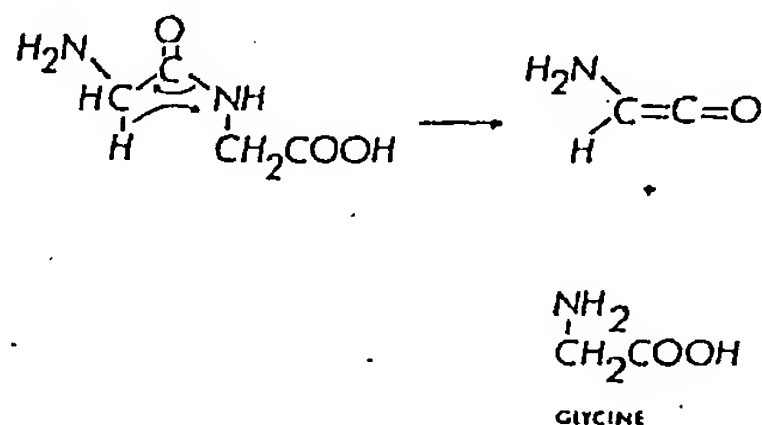
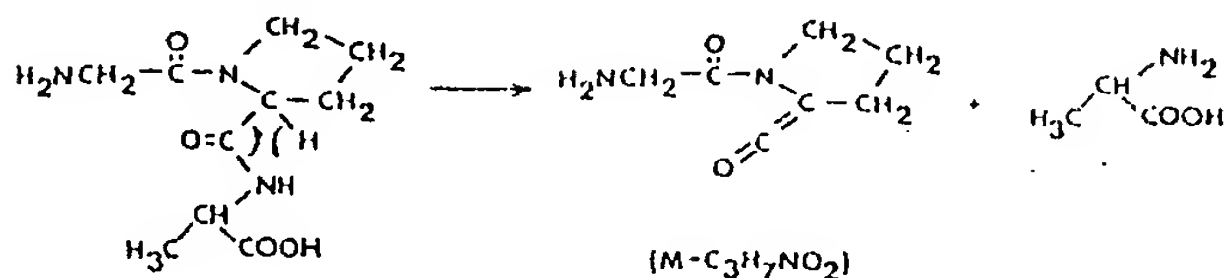


Figure 10. K⁺ CI/surface ionization spectra of three representative peptides. Heating current was taken rapidly from 0 to 2 A before spectra were recorded.

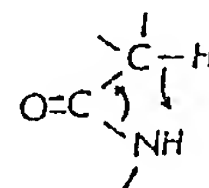
10 shows representative spectra of a dipeptide and two tripeptides. Note that in each case an (M + K)⁺ is observed. For each fragment, bond cleavage with a H shift can explain the observed species. For example, glycylglycine (Gly-Gly) can thermally decompose to form glycine.



The spectra of glycylglycine obtained in this way is fairly simple. A similar process can be postulated for the tripeptide glycyl-L-prolyl-L-alanine (Gly-Pro-Ala), leading to the product *m/z* 193.



Following this decomposition, K⁺ complexes with these surface species to produce [(M - C₃H₇NO₂) + K]⁺ at *m/z* 193. A similar process generates the *m/z* 226 product for Leu-Gly-Phe. Note that in all three cases, cleavage of the peptide linkage



dominates the decomposition pathways. Thus, the possible use of this technique for sequencing such species is being investigated. Note that DL-leucylglycyl-DL-phenylalanine (Leu-Gly-Phe) has a much more complex spectrum at lower masses. Many of the products do not contain K⁺. Apparently, some bases are more susceptible to surface ionization. This behavior is being investigated in more detail.

Over a dozen di- and tripeptides have been successfully analyzed by using this technique. In all cases, an (M + K)⁺ was observed, with (M + K - 18)⁺ and (M + K - 44)⁺ ions being common as well. Some compounds exhibit bias dependent spectra. For example, L-methionyl-L-phenylalanine (Met-Phe) has a simple spectrum ((M + K)⁺, (M + K - CO₂)⁺) at 2.5 V bias but becomes more complex at 3.0 V bias, approaching previously published spectra (22).

In some ways, this approach to analysis of nonvolatile compounds may be preferable to alternate methods such as FAB ionization, for a number of reasons. The most obvious advantage is cost. Secondly, the absence of a matrix and its associated spectral interference, e.g., the glycerol matrix in FAB, is an advantage. Also, FAB frequently only produces an adduct ion of an analyte, (M + H)⁺, with little structural information. Presumably, if this experiment could be performed with a K⁺ bead which emitted K⁺ with *no* heating, peptides would only show an (M + K)⁺ ion. The temperature at which K⁺ emission occurs appears to induce low-energy decompositions of such compounds to smaller molecules. The K⁺ then samples these to generate simple, useful spectra. In some ways the technique is analogous to FAB or SIMS—the major difference being that K⁺ is generated from the surface on which the species is deposited.

CONCLUSIONS

A number of uses for a hot glassy bead which generates metal ions are discussed. For most compounds, addition processes are observed, if the kinetic energy of K⁺ is (relatively) low. If this energy is raised, addition will not occur. Some compounds, notably amines, react by surface ionization. Higher biases on the emitter can produce these ions. This gives the basis for a novel CI method, which can also be used to selectively ionize certain compounds in a mass spectrometer source. Also, when the emitter is coated with larger, nonvolatile molecules, fragments are produced which are representative of the molecule and complexed to K⁺. This may be a simple alternative to other ionization methods such as FAB. As thermionic sources for transition-metal ions are developed, kinetic energy dependent CI should be a useful tool for obtaining functional group and structural information.

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Elemental Isotopic Abundance Determinations of Magnesium in Biological Materials by Secondary Ion Mass Spectrometry

G. O. Ramseyer, J. T. Brenna, and G. H. Morrison*

Department of Chemistry, Cornell University, Ithaca, New York 14853

Ruth Schwartz

Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853

Secondary ion mass spectrometry is applied to the determination of stable magnesium isotopes. The method involves the high mass resolution determinations of the isotopes from air-dried films of untreated and acid-treated biological fluids. Calibration curves are used for the isotopic quantifications. Agreements of less than 2.5% enrichments of ²⁶Mg were determined for spiked and unspiked acid-treated samples. Differences of about 5%, 2%, and 1% enrichments can be determined with 95% certainty in analyses times of 2, 15, and 30 min, respectively.

Secondary ion mass spectrometry (SIMS) is a sensitive technique capable of determining elemental isotopes in a broad range of solid materials. The accuracy of many low mass resolution SIMS isotopic determinations is affected by molecular interferences. SIMS instruments such as the Cameca MS-3F ion microanalyzer are capable of resolving isotopic peaks which require mass resolutions ($M/\Delta M$) of over 10 000 to separate. The isotopic abundance measurements of elements in pure metals (1-4), geological materials (4-7), and tissues (4, 8, 9) are affected by instrumental parameters and matrix effects. High mass resolution SIMS has not been applied to the quantification of elemental isotopic abundances in biological materials.

Because of the shortage of suitable radioisotopes and restrictions on their use for human biomedical research, there has been an increase in the application of stable isotopes as biological tracers (10). The functions of magnesium in the body include maintenance of membrane integrity, muscular contraction and contractability, transmission of nerve impulses, and

regulation of responses to hormones. Despite its importance, research on magnesium has lagged behind that of other essential elements. One of the reasons for this has been the lack of a suitable tracer isotope. The only radiotracer available is the short-lived γ -emitter ²⁸Mg ($T_{1/2} = 21.3$ h), which is inconvenient and too short-lived for complete whole body magnesium turnover studies.

Analytical methods applicable to stable isotopic abundance measurements in biological materials have been reviewed (11, 12). Although a few stable isotopes can be determined by neutron activation analysis, mass spectrometric methods can determine all isotopes. Thermal ionization mass spectrometry has been shown to yield measurements of high accuracy and precision (13, 14); however sample preparation and analysis are time-consuming and not applicable to large numbers of samples. SIMS has the potential of providing rapid routine analysis of small sample volumes with a minimum of sample preparation. Wittmaack (15) has developed methodology for determining elemental isotopic abundances in whole blood by low mass resolution SIMS. However, molecular interferences and sample charging were observed. Burns (16) has discussed the kinetics of uptake in biological tissue by use of stable isotopes and secondary ion microscopy. Quantitative results from this work have not been presented.

The purpose of this study was to investigate the precisions, accuracies, and time requirements for low and high mass resolution SIMS determinations of stable isotopes in biological materials. General analytical procedures such as the use of calibration curves are evaluated. The importance of sample homogeneity and its effect on the precision of SIMS isotopic determinations are discussed. Acid digestion procedures are presented for the analysis of biological materials which, be-